

Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCM1



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ARTICLE INFO

Article history:

Received 23 December 2015

Accepted 23 December 2015

Available online 31 December 2015

ABSTRACT

The human embryonic stem cell line RCM-1 was derived from a failed to fertilise egg undergoing parthenogenetic stimulation. The cell line shows normal pluripotency marker expression and differentiation to three germ layers *in vitro* and *in vivo*. It has a normal 46XX female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.

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Resource table

Name of stem cell construct	RCM1
Institution	Roslin Cells Ltd. St Mary's Hospital, Manchester
Person who created resource	B. Tye, J. Gardner, C. Pope, S. Sneddon, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, K. McDonald, H. Bradburn
Contact person and email	Paul.desousa@roslincells.com ; Paul.desousa@ed.ac.uk ; Janet.downie@roslincells.com Aidan.courtney@roslincells.com daniel.brisson@manchester.ac.uk
Date archived/stock date	21 December 2006 (passage 10 on feeders) 07 February 2011 (seed bank at passage 24)
Type of resource	Biological reagent: Cell line
Sub-type	hESC, research grade
Origin	Blastocyst with ICM and trophoblast originating from clinically failed egg receiving parthenogenetic stimulus. Confirmed as biparental by whole genome SNP analysis.
Key transcription factors	Oct4 (confirmed by flow cytometry and immunocytochemistry)
Authentication	See Quality Control Test Summary, Table 1
Link to related literature (direct URL links and full references)	De Sousa PA*, Gardner J, Sneddon S, Pells S, Jorgenson Tye B, Dand P, Collins DM, Steward K, Shaw L, Przyborski S, Cooke M, McLaughlin KJ,

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Resource table (continued)

Name of stem cell construct	RCM1
	Kimber SJ, Lieberman BA, Wilmot I, Brison D. (2009) Clinically failed eggs as a source of normal human embryo stem cells. <i>Stem Cell Research</i> 2, 188–197. http://www.sciencedirect.com/science/article/pii/S1873506109000026
Information in public databases	http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/cell_lines.aspx
Ethics	Informed consent obtained. Approved by participating institutional ethics advisory boards and Central Manchester Local Research Ethics Committee reference number 03/CM/684. Conducted under the UK Human Fertilisation and Embryology Authority licence no R0136.

Resource details.

RCM1 was derived from a failed to fertilise egg subjected to parthenogenetic activation. The cell line was derived by whole embryo outgrowth on mitotically inactivated human fibroblast (HDF) feeder cells using HDF conditioned medium and expanded under feeder free conditions.

RCM1 was shown to be pluripotent by expression of Oct 4 using immunocytochemistry ([Table 1](#), [Fig. 1](#)). By flow cytometric analysis, the expression of pluripotency makers Oct4, Tra-1-60 and SSEA-4 was 97%, 98% and 99%, respectively, whereas low expression of the differentiation marker SSEA-1 (6%) was observed ([Fig. 2](#)).

Table 1
Summary of quality control testing and results for RCM-1.

Classification	Test	Purpose	Result
Donor screening	HIV 1 + 2 Hepatitis B Hepatitis C	Donor screening for adventitious agents	Negative
Identity	Microsatellite PCR (mPCR)	DNA Profiling to give cell line its signature, gender/species	Performed
Phenotype	Immunocytochemistry	To assess levels of staining for the pluripotency markers Oct4 and Nanog	Expression of Oct4 and Nanog
	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	Oct3/4: 97% Tra 1-60: 98% SSEA-4: 99% SSEA-1: 6%
Genotype	Blood group genotyping (DNA analysis)	To establish blood group of the line	AA
Microbiology and virology Morphology	Karyology (G-banding)	Confirmation of normal ploidy by G-banding	46XX
	HLA tissue typing	To establish full HLA Type I and Type II genotypes of the line	HLA typed Class I and Class II
	Mycoplasma Photography	Mycoplasma testing by RT-qPCR To capture a visual record of the line	Negative Normal
Differentiation potential	Embryoid body formation	To show differentiation to three germ layers	Expression of muscle actin, β -tubulin and α -feto protein
	Teratoma formation	To show differentiation to three germ layers	Teratoma formation observed, three germ layers detected

Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation and teratoma formation (Fig. 1).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and Class II typing is available (Table 1). Blood group genotyping gave the blood group AA (Table 1). The cell line was analysed for genome stability by G-banding and GCH (Fig. 3), as well as a SNP array screen to determine whether RCM1 was derived from a parthenogenetic or fertilized embryo. This showed a normal, 46XX female genotype with no genetic deficiencies that had originated from a fertilized egg.

The cell line is free from mycoplasma contamination as determined by RC-qPCR.

Materials and methods

Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A Research Ethics Committee and local ethics board at participating fertility clinics and conducted under licence no R0136 from the UK HFEA with informed donor consent.

Cell culture

Activation and derivation of RCM1 is described in De Sousa et al. (2009), Stem Cell Research 2, 188–197. The established cell line was expanded and banked using CellStart matrix and Stempro hESC Serum Free Medium (ThermoFisher Scientific (Life Technologies), Paisley, UK). Passaging was performed mechanically using an EZ passage tool (ThermoFisher Scientific).

Mycoplasma

Mycoplasma detection was performed using Applied Biosystems PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit and MicroSEQ™ Mycoplasma Real-Time PCR Detection Kit

(ThermoFisher Scientific (Applied Biosystems)) according to the manufacturer's instruction.

Flow cytometry

Pluripotency was determined using the Human and Mouse Pluripotent Stem Cell Analysis kit (BD, Oxford, UK). Oct3/4 and SSEA-4 were included as pluripotency markers, and SSEA-1 as a differentiation marker. FITC conjugated Tra-1-60 (BD) was used as an additional pluripotency marker. Fixed and permeabilised cells were analysed using the Guava easyCyte flow cytometer (Millipore, Watford, UK).

Immunocytochemistry

hESC were fixed in 4% paraformaldehyde (ThermoFisher Scientific (Alfa Aesar)), permeabilised using 100% ethanol (ThermoFisher Scientific) and stained with AFP (1:500; Sigma Aldrich, Dorset, UK), β -tubulin III (1:1000; Sigma), muscle-specific actin (1:50; DAKO, Glostrup, Denmark), Oct4 (1:200; Santa Cruz Biotechnology, Texas, USA), Nanog (1:20; R&D Systems, Abingdon, UK) and secondary antibodies anti-mouse IgG-FITC (1:200; Sigma), anti mouse IgG – AlexaFluor 488, anti-goat IgG – AlexaFluor 488 and anti-goat IgG – AlexaFluor-594 (1:200 donkey polyclonal AlexaFluor-594) (all 1:200; ThermoFisher Scientific). Alkaline Phosphatase staining was as per manufacturer's recommended protocol (Vector labs, Peterborough, UK). Images were acquired using a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope.

In vitro differentiation

hESC cells were pre-treated for 1 h with 10 μ M ROCK inhibitor in Stempro hESC SFM (ThermoFisher Scientific) and embryoid bodies EBs generated in ultra low attachment plates (Corning) for 7 days before being transferred into EB medium (20% FBS (GE Healthcare (PAA), Buckinghamshire, UK), 80% KO-DMEM 1 mM L-glutamine, 0.1 mM β -mercaptoethanol, 1% nonessential amino acids (all ThermoFisher Scientific)), on glass slide tissue culture chambers

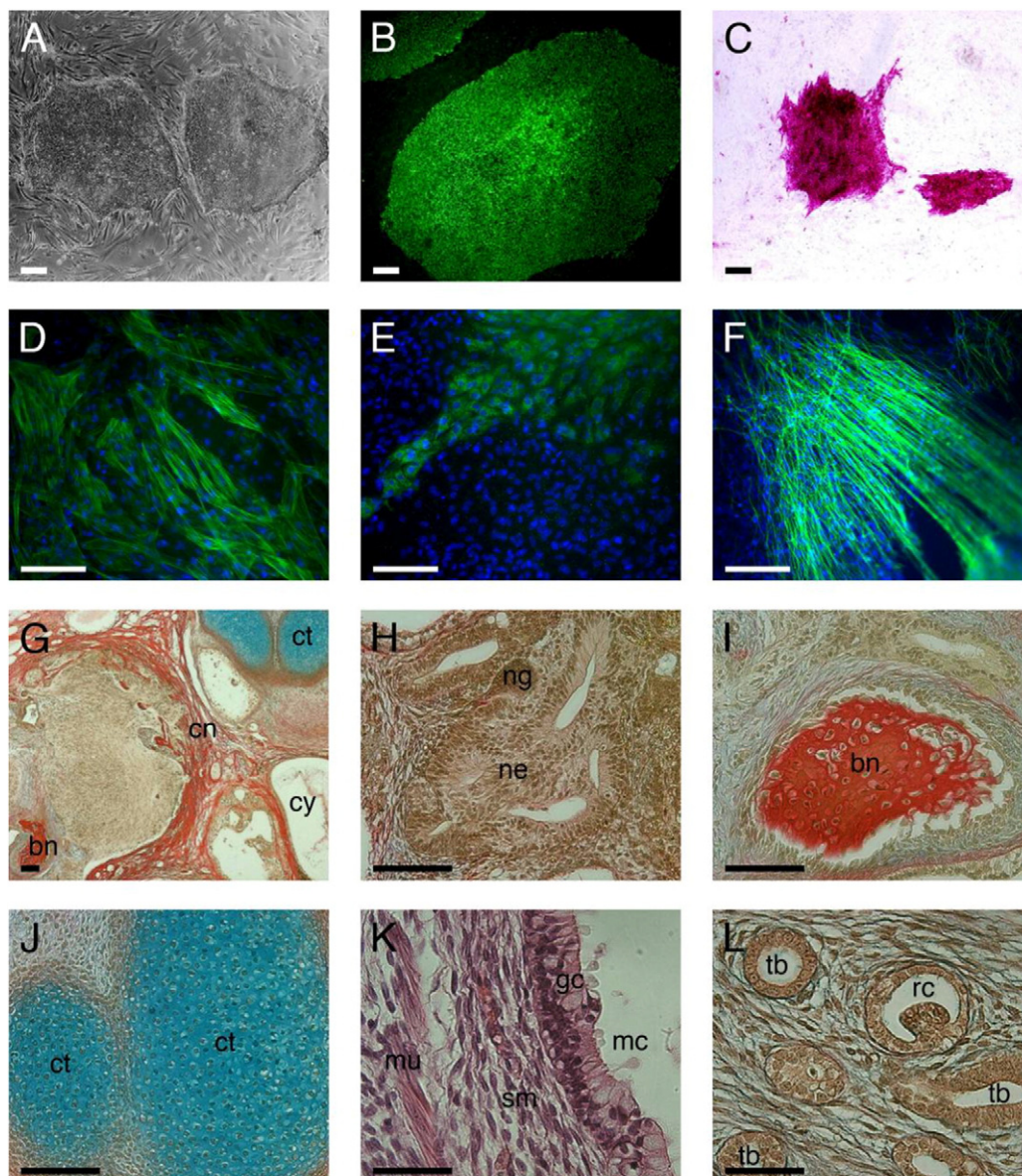


Fig. 1. The characterization of RCM1 as a pluripotent hESC line. Undifferentiated colonies of RCM1 on human dermal fibroblast feeder cells are shown (A) in phase contrast, (B) stained positively for Oct4 and (C) stained positively for alkaline phosphatase activity. RCM1 cells *in vitro* differentiated via embryoid bodies and stained for markers of the three germ layers: (D) muscle-specific actin (mesoderm), (E) α -fetoprotein (endoderm), and (F) β -tubulin III (ectoderm). A histological assessment was made of teratoma tissue formed as a result of transplantation of the hESC line, RCM1, into the testis of adult SCID mice. (G) Low-power image showing heterogeneity of tissue structure in tumor. Identifiable structures include bone (bn), cartilage (ct), connective tissue (cn), and small cysts (cy); higher power images show (H) neuroectoderm (ne) and neural ganglion (ng) and (I) bone (bn). (J) Cartilage; (K) wall of primitive intestine, including muscular layer (mu), submucosal tissue (sm), and goblet cells (gc) within epithelium releasing mucus secretion (mc); (L) primitive kidney consisting of renal corpuscle (rc) and associated tubules (tb). (G–J, L) Weigert's and (K) hematoxylin and eosin. Scale bars, 100 μ m (A–J) and 50 μ m (K and L). From De Sousa et al. (2009).

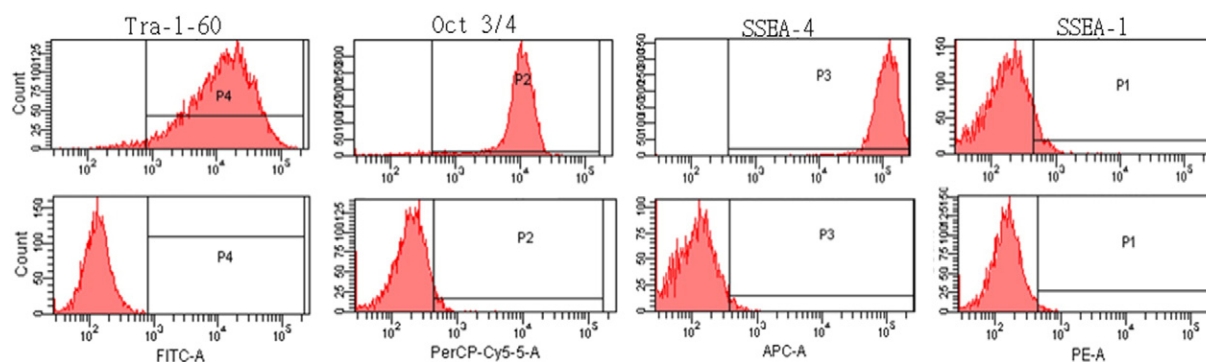


Fig. 2. Flow cytometry analysis of undifferentiated RCM1. RCM-1 was subjected to flow cytometry analysis for markers of pluripotency. The specific antibody stained histograms are shown in the top row against the corresponding control histograms. Percentage staining is indicated in Table 1.

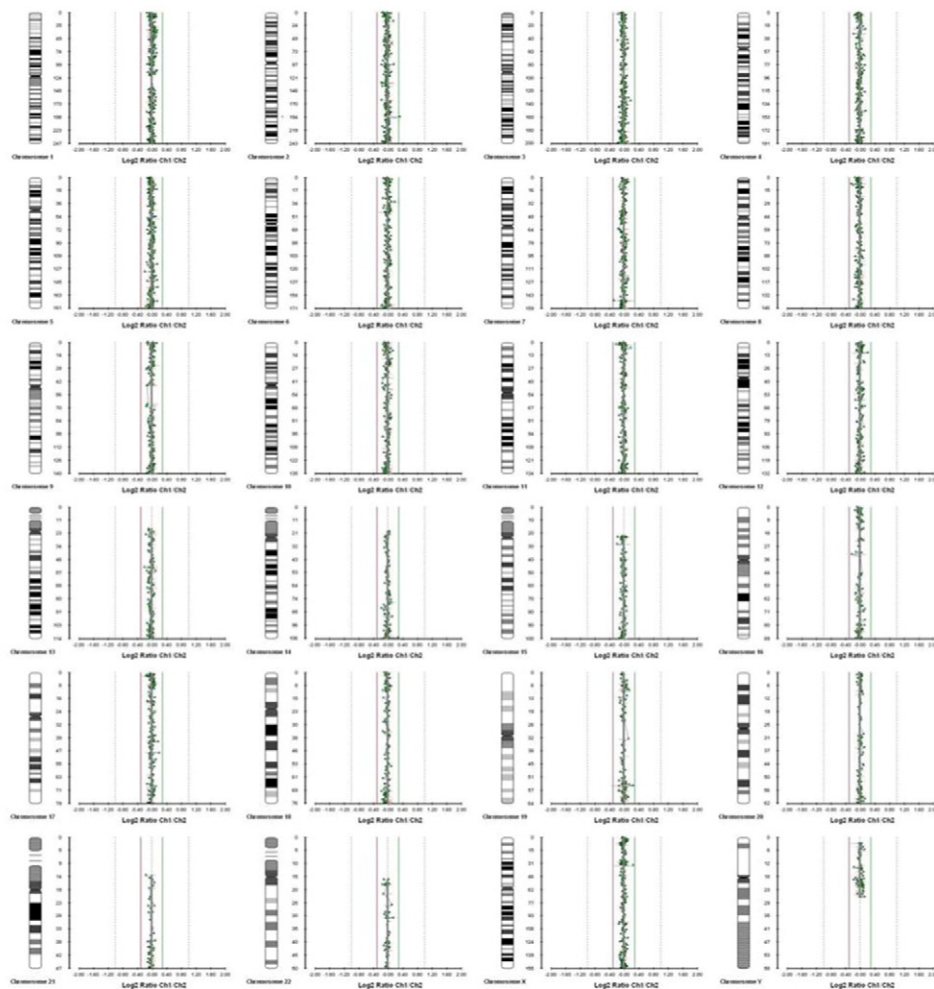
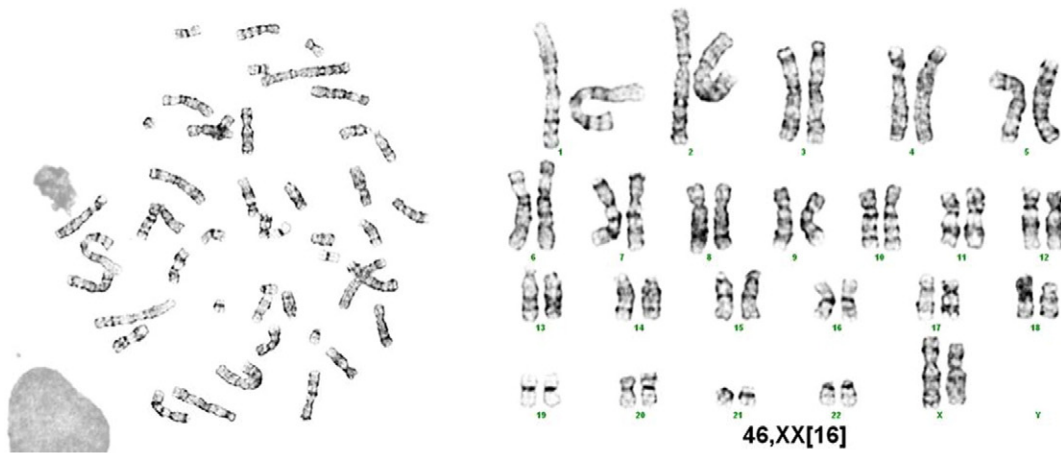


Fig. 3. Karyotype analysis of RCM-1. RCM-1 was analysed by Giemsa staining of 20 metaphase spreads and showed a normal 46XX female karyotype (top panel). No genomic copy number imbalance was evaluated using a BlueGnome CytoChips array designed to improve detection of copy number variation associated with constitutional genetic disorders (bottom panel). Presented are SNP polymorphism copy number representation which did not exceed normal ranges (flanking green and red lines) depicted next to chromosome ideograms. From De Sousa et al. (2009).

(Nunc, ThermoFisher Scientific) coated with 0.5% gelatin (Sigma) at 0.1 ml/cm² for 14 days.

In vivo differentiation

The developmental potential of RCM-1 to form teratomas consisting of tissues representative of all three germ layers was evaluated

following transplantation under kidney capsule in adult SCID mice and teratoma formation assessed as described in [De Sousa et al. \(2009\)](#), Stem Cell Research 2, 188–197.

Genomic analysis

All outsourced assays were carried out under a Quality and Technical Agreement. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Manchester, UK) according to the manufacturer's recommendations.

Microsatellite PCR, or Short Tandem Repeat analysis, was used to determine cell line identity and was carried out by Public Health England. A profile was obtained for the following core alleles: vWA, D16S539, Amelogenin, TH01, CSF1PO, D5S818, D7S820, D13S317 and TPOX.

Human Leukocyte Antigen (HLA) tissue typing was carried out by the Scottish National Blood Transfusion Service.

Blood group genotyping was carried out by the Molecular Diagnostics laboratory at NHSBT.

Karyotype analysis was carried out by The Doctors Laboratory (London, UK) or the Western General Cytogenetics Laboratory (Edinburgh, UK) Live cells at 60–70% confluency were shipped overnight in warm containers, fixed and analysed by standard G-banding analysis. For research grade lines, 20 spreads were analysed.

Comparative genome hybridization (CGH) was carried out in collaboration with ARK Genomics (Roslin Institute, UK), using BlueGnome CytoChipTM arrayCGH v2. Genome-wide SNP analysis genomic DNA

was carried out with GeneChip Human Mapping 250 K Sty I and 250 K Nsp arrays (Affymetrix), both as described in [De Sousa et al. \(2009\)](#), Stem Cell Research 2, 188–197.

Verification and authentication

The identity and phenotype of RCM1 was confirmed by quality control testing using the aforementioned methods the results of which are summarised in [Table 1](#), [Figs. 1 & 2](#). Further information on this line can be obtained from its original publication in [De Sousa et al., 2009](#).

Acknowledgements

Research culminating in the derivation of this line was funded by a grant from the UK Medical Research Council (G0300484) to PDS and DRB, and Scottish Enterprise Economic Development Agency (PM07321) to PDS and AC.

References

- De Sousa, P.A., Gardner, J., Sneddon, S., Pells, S., Jorgenson Tye, B., Dand, P., DM, Collins, Steward, K., Shaw, L., Przyborski, S., Cooke, M., KJ, McLaughlin, SJ, Kimber, BA, Lieberman, Wilmut, I., Brison, D., 2009. [Clinically failed eggs as a source of normal human embryo stem cells](#). *Stem Cell Res.* 2, 188–197.